A Promoter Sequence Variant of *ZNF750* Is Linked with Familial Psoriasis

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We previously mapped a psoriasis-susceptibility gene to a 3.8-Mb region of the 17q terminus in a five-generation Chinese family with autosomal-dominant psoriasis. To identify the mutations responsible for the psoriasis in this family, we sequenced 78 genes within the region and found four gene variants, p.Ala201Val in *CD7*, c.−625A>C in zinc-finger protein 750 (*ZNF750*), p.Asp189Asn in *C17orf56*, and p.Ala568Thr in *AATK* cosegregated with the disease. The latter two variants were not studied further in the absence of disease segregation in other familial psoriasis and presence of variants in normal subjects. Functional analyses of CD7 did not support *CD7* as a disease-causing gene. In contrast, the c.−625A>C mutation in *ZNF750* resulted in a 42% reduction of the promoter activity, and the electrophoretic mobility shift assay showed binding of nuclear protein(s) to the mutant C allele. The c.−625A>C mutation was found in another sporadic psoriasis patient but was absent in 188 normal controls. Together, the mutation accounts for 1.7% (confidence interval: 0.2–5.84%) of psoriasis in the Chinese population. This report suggests that *ZNF750* mutations could contribute to psoriasis susceptibility.

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INTRODUCTION

Psoriasis is a chronic inflammatory disorder of the skin, nails, scalp, and joints, which varies in severity and clinical manifestation. The most common form, plaque psoriasis, affects 2–3% of the Caucasian population (Lebwohl, 2003) but only 0.3% of the Mongoloid population (Yip, 1984). The skin lesions present with hyperproliferation and incomplete differentiation of epidermal keratinocytes, an increase in dermal capillaries, and marked infiltration of T cells and neutrophils in the dermis and epidermis. Current evidence suggests that psoriasis is an immune-mediated disorder and novel therapies involved in the suppression of the immune responses, such as the T-cell-targeted agents and tumor necrosis factor (TNF) inhibitors, have improved the outcome of the disease (Krueger, 2002; Gottlieb, 2005). However, not all patients respond to these therapies and the efficacy varies between psoriasis patients.

A number of genetic studies have been performed to identify the psoriasis-susceptibility loci. At least nine candidate loci have been reported (PSORS1–9) (Bowcock and Cookson, 2004), indicating that psoriasis is inherited as a complex trait with several genes involved and that it can be influenced by environmental factors. Except for the most repeatedly mapped HLA class I region (PSORS1), many groups, including our own, have demonstrated linkage or association of familial psoriasis to a locus on 17q25 (PSORS2) (Tomfohrde *et al.*, 1994; Helms *et al.*, 2003; Capon *et al.*, 2004; Hwu *et al.*, 2005; Stuart *et al.*, 2006). A RUNX1 binding site variant between *SLC9A3R1* and *NAT9* on chromosome 17q25 has been identified as a psoriasis-susceptibility gene from a family-based association study (Helms *et al.*, 2003). Another locus within the *RAPTOR* gene (regulatory associated protein of MTOR) has also been implicated in psoriasis susceptibility (Helms *et al.*, 2003). Recently, a gene of unknown function, zinc-finger protein 750 (*ZNF750*), lying on the distal end of chromosome 17q was found to associate with a seborrhea-like dermatitis with psoriasiform elements (Birnbaum *et al.*, 2006). ZNF750 (OMIM# 610226) is a putative member of the zinc-finger transcription factors with a nuclear localization site at the 5′ end and a conserved C_2H_2 zinc-finger domain. *ZNF750* is expressed in skin, thymus, prostate, lung, and placenta, with the highest amount in keratinocytes. The skin biopsies of the patients showed some of the pathological elements seen in psoriasis, suggesting the involvement of ZNF750 in the pathogenesis of psoriasis.

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Abbreviations: CI, confidence interval; EMSA, electrophoretic mobility shift assay; PBMC, peripheral blood mononuclear cell; SNP, single-nucleotide polymorphism; TNF, tumor necrosis factor; ZNF750, zinc-finger protein 750

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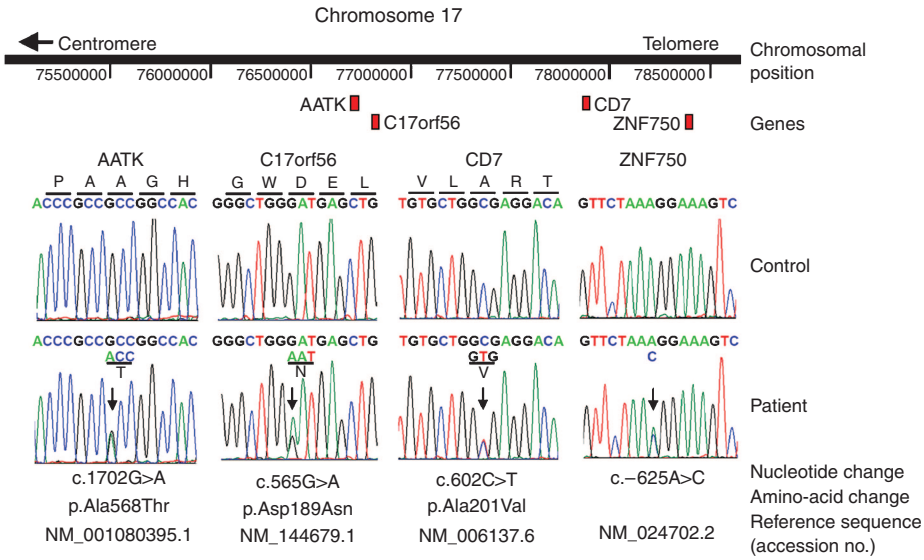

Figure 1. Schematic representation showing four variants cosegregated with the disease in the family. The position and size of the genes are indicated by red squares. Nucleotide sequence and amino-acid translation of the patients show heterozygous variation marked by arrows.

In our previous study of a five-generation extended psoriasis family, we mapped a psoriasis-susceptibility locus to a 3.8-Mb region of the chromosome 17q terminus using a genome-wide scan. Here, we report further investigation of candidate genes in this region as well as identification and characterization of sequence variants in two of the candidate genes in Chinese patients with psoriasis.

RESULTS

Sequence of candidate genes on chromosome 17q terminus

We have previously mapped and narrowed down the psoriasis-susceptibility locus in our index family to a 3.8-Mb region between D17S1806 and the 17q terminus of chromosome 17 (Hwu *et al.*, 2005). This candidate interval contains 94 genes, including 44 genes of unknown function. We sequenced 78 genes, including all genes known to be expressed in skin and/or immune cells as per the HMDEG (Human and Mouse Differentially Expressed Genes) database (http://gln.ibms.sinica.edu.tw/product/HMDEG/EST/index.php) and microarray expression data in the UCSC (University of California Santa Cruz) database (http://genome.ucsc.edu/). The remaining 16 genes were not sequenced primarily because they have (1) limited tissue distribution, such as being expressed only in the liver, eye, muscle; or (2) housekeeping genes with mutations resulting in known phenotypes/diseases; or (3) hypothetical genes with unknown tissue distribution. In addition, we sequenced for RUNX1 binding site variant (rs734232, G>A) previously inferred to be psoriasis-susceptibility gene 5 Mb proximal to our candidate locus and another psoriasis-associated single-nucleotide polymorphism (SNP) (rs869190, G>T) in the raptor gene within our locus on chromosome 17q. Our proband had G allele present in both RUNX1 binding site variant and the psoriasis-associated SNP of the raptor gene.

A total of 22 sequence variants present in the proband, but not in the normal control, and not found in the dbSNP databases were subjected to segregation analysis, which consisted of sequencing all 23 affected members (13 severely affected and 10 mildly affected) and 16 unaffected members for the portion of the genes containing variants. Four genes with sequence variants cosegregated with the disease in our five-generation index family were identified: they were p.Ala201Val in the *CD7* gene, p.Asp189Asn in the *C17orf56*, p.Ala568Thr in the *AATK*, and c.−625A>C in the *ZNF750* gene (Figure 1). To our knowledge, these variants are all previously unreported. The p.Asp189Asn variant of *C17orf56* and p.Ala568Thr variant of *AATK* did not segregate with the disease in other familial psoriasis families (one in *C17orf56* and two in *AATK*). Furthermore, the allele frequencies of the two variants in psoriasis patients (2.83% for *C17orf56* and 2.4% for *AATK*) did not differ from the controls (1.97% for C17orf56 and 1.09% for AATK) ($P=0.56$ and $P=0.39$ for *C17orf56* and *AATK*, respectively). Therefore, these two gene variants were not studied further.

The p.Ala201Val variant in *CD7* showed complete disease segregation in familial psoriasis and was present in 1.38% (3 of 109) of psoriasis patients. However, it was also detected in two normal individuals (2 of 161 Han Chinese controls, allele frequency 0.62%) ($P=0.4$, cases versus controls); one has atopic dermatitis and the other is allergic to seafood. The c.−625A>C variant of *ZNF750* also segregated with the disease in familial psoriasis and was completely absent in all 188 normal Han Chinese subjects tested. These data suggested that *CD7* and *ZNF750* act as putative genes for psoriasis in this family. The functional consequences of the *CD7* and *ZNF750* mutations were further investigated.

Analysis of the *CD7* p.Ala201Val variant

Human CD7 (OMIM# 186820) is a surface membrane-associated glycoprotein expressed mainly on the surface of human T and NK cells and, similar to CD28, CD7 acts as a costimulatory molecule (Stillwell and Bierer, 2001). The

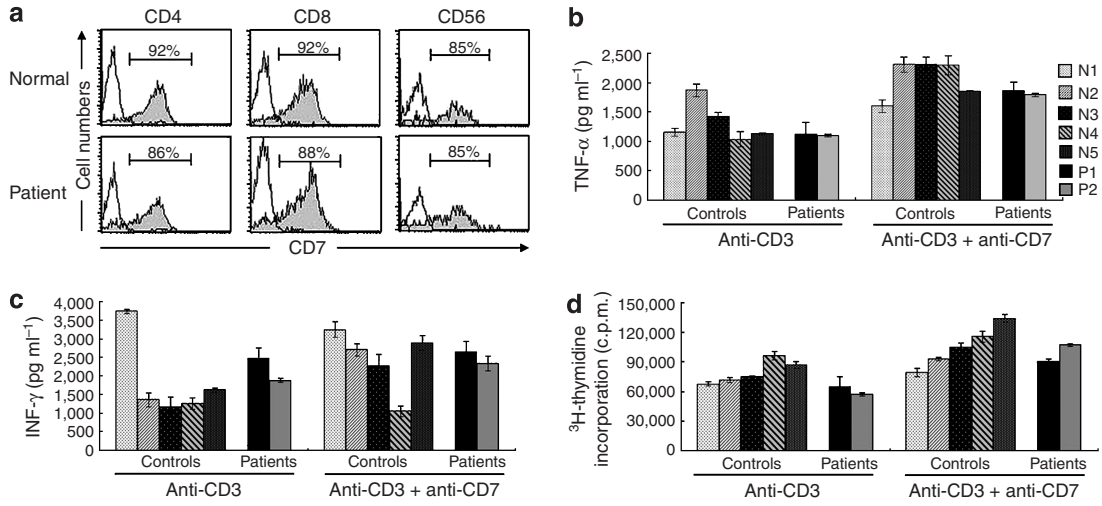

Figure 2. The effect of p.Ala201Val variant in CD7. (**a**) Flow cytometric analysis of CD7 expression in gated CD4+ T cells, CD8+ T cells, and CD56+ NK cells from a representative normal control (upper panel) and his affected brother (lower panel). Histograms show surface fluorescence intensity of isotype-matched control (empty histogram) and anti-CD7 mAb (filled histogram). The percentage of CD7 expression was indicated. (**b**, **c**) PBMC cells from two patients (P1, P2) and five normal controls (N1–N5) were cultured with anti-CD3 or anti-CD3 plus anti-CD7 in a total volume of 200 µl. The 2-day culture supernatants were collected and analyzed for production of (**b**) TNF-α and (**c**) IFN-γ by ELISA. (**d**) PBMC cells from two patients and five normal controls were cultured with anti-CD3 or anti-CD3 plus anti-CD7, and cell proliferation was performed by ^{3}H-thymidine uptake.

p.Ala201Val mutant identified in the *CD7* gene is located in the predicted transmembrane domain of the CD7 molecule. To investigate if the mutation could affect the expression of CD7, we isolated peripheral blood mononuclear cells (PBMCs) from two patients and five controls and analyzed the CD7 expression on T and NK cells by using flow cytometry. The expression level of CD7 and the percentage of CD7-positive cells in CD4, CD8, or CD56 (NK) cells showed no difference between patients and controls; a representative set of data was shown in Figure 2a. We next examined whether the mutation affects the functionality. We measured the costimulatory activity of CD7 by evaluating the capability of cytokine production and T-cell proliferation in response to anti-CD3 and anti-CD3/CD7 mAb. As expected, cell costimulation with anti-CD7 antibody further increased the production of IFN-γ and TNF-α and proliferation ability as compared with stimulation with anti-CD3 alone. However, when compared with normal controls, PBMCs from two psoriasis patients produced similar levels of IFN-γ and TNF-α and proliferation ability when cultured with anti-CD3 in the presence or absence of CD7 mAb over a time course of 12, 24, 48, and 72 hours. A representative data set of 48-hour culture was shown in Figure 2b–d. These data showed that *CD7* is unlikely to be a disease-causing gene.

ZNF750 c.−625A>C mutation decreases *ZNF750* promoter activity

The second candidate gene sequence variant identified in the index family was located 625 bp upstream from the start codon of the *ZNF750* gene (c.−625A>C). Alignment of the 5′ genomic DNA sequence of *ZNF750* from human, macaque, mouse, opossum, dog, cow, and chicken showed that the c.−625A is conserved in all these species except the cow (http://www.ebi.ac.uk/Tools/clustalw/index.html). No other sequence variations of the *ZNF750* gene were found in this family.

Computer searches of transcription factor binding sites revealed that the c.−625A>C substitution eliminated a potential NF-AT site and introduced overlapping c-Myb and c-ETS sites. To investigate whether this nucleotide substitution would affect the expression of *ZNF750*, we constructed *ZNF750* promoter fragments containing the variant. We performed the promoter assay in a keratinocyte cell line, HaCaT, because the highest expression of *ZNF750* is in keratinocytes. The c.−625C promoter showed an approximately 42% decrease in transcriptional activity compared with the wild-type promoter (Figure 3).

We further investigated whether the downregulation of *ZNF750* by the c.−625A>C variant was due to the loss or gain of transcription factor binding. Electrophoretic mobility shift assay (EMSA) showed a strong binding of nuclear protein(s) to the oligonucleotide with the mutant C allele as compared to the wild-type A allele (Figure 4a, lanes 1–4). This DNA–protein complex was inhibited in a dose-dependent manner by the unlabeled mutant C allele, but much less so by the wild-type A allele (Figure 4a, lanes 5–10), further suggesting the specificity of nuclear protein(s) binding to the mutant allele containing C at the c.−625 locus. Competitive assays showed that consensus sequences recognized by ESE-1, ESE-2, and PU.1 (Figure 4b, lane 2) competed more efficiently for the DNA–protein complex than the sequences recognized by other transcription factors, including PDEF, ETS1, NF-κB, NF-AT, and c-Myb (Figure 4b, lanes 3–8).

ZNF750 sequence variations in other patients with psoriasis

The c.−625A>C variant was not found among the 35 other familial psoriasis patients but was found in one of the 85

sporadic psoriasis patients studied (Table 1). None of the 188 normal subjects contained this mutation. This c.−625A>C variant accounts for 1.7% (2 of 121, confidence interval (CI): 0.2–5.84%) of the psoriasis in the Chinese population.

To further study *ZNF750* variations in psoriasis, we sequenced the *ZNF750* gene in 85 sporadic psoriasis patients as well as 35 additional index patients, each from a family with familial psoriasis. The same c.−625A>C variant was found in a sporadic psoriasis patient, a c.−646G>A variant in one family with familial psoriasis, a c.−597C>T variant in a sporadic psoriasis patient, and a missense variant of c.1046T>C (p.Leu349Pro) was detected in another family with familial psoriasis (Table 1). None of these variants were found in any of the 188 normal controls. The functional significance of these variants remained to be investigated.

In addition to the unique sequence variants found only in psoriasis, a c.1305C>G (p.Asn435Lys) was found in 2 familial psoriasis and 13 sporadic psoriasis cases; however, this single-nucleotide change is likely to be a polymorphism, as it was also present in high frequency in normal controls (6.9%, 13 of 188) (Table 1).

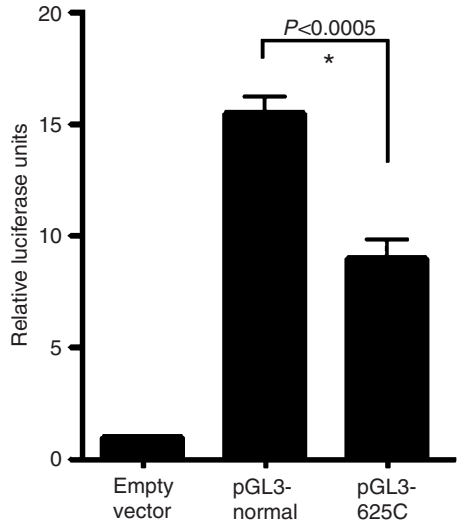

Figure 3. The effect of the variations in the promoter region of *ZNF750*. The luciferase activity of the reporter constructs containing either the A or the C allele at the c.−625 locus in HaCaT cells. The results represent means ± SD of five independent experiments. *Student's *t*-test.

DISCUSSION

In this study, we used the classical positional cloning approach by sequencing the candidate genes in the 17q region to identify the responsible gene/mutation for psoriasis in this unusual five-generation family. Those sequence variants present in the proband, but not in the normal

Table 1. Frequency of *ZNF750* sequence variants in affected individuals and normal controls that were first identified in psoriasis patients

Variants[1]	Familial psoriasis (*n*=36)	Sporadic psoriasis (*n*=85)	Normal controls (*n*=188)
c.−625A>C	1	1	0
c.−646G>A	1	0	0
c.−597C>T	0	1	0
c.1046T>C (p.L349P)	1	0	0
c.1305C>G (p.N435K)	2	13	13

n, number of individuals; ZNF, zinc-finger protein.
[1]The *ZNF750* cDNA reference sequence used was under GenBank accession number NM_024702.2. The numbering is based on +1 as the A of the ATG translation initiation codon.

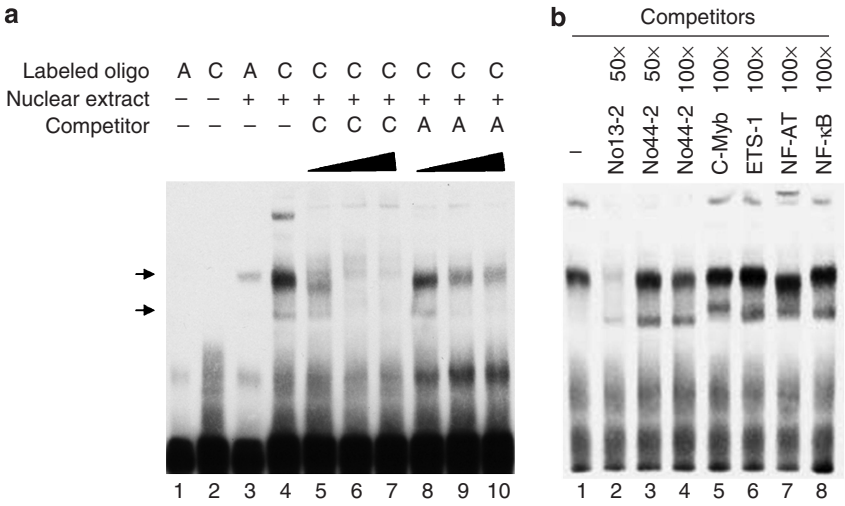

Figure 4. EMSA showing binding of transcription factors to the −625C allele. (**a**) EMSAs were performed with HaCaT nuclear extract and the biotin-labeled 30-bp oligonucleotides containing the normal allele A or mutant allele C (lanes 1–4). Competition experiments were performed by using biotin-labeled oligonucleotides with mutant alleles and a 5-fold (lanes 5 and 8), 50-fold (lanes 6 and 9), and 100-fold (lanes 7 and 10) excess of unlabeled mutant allele (lanes 5–7) or normal allele (lanes 8–10). Arrows indicate the DNA–protein complex. (**b**) Competition assays. Lane 1 contained biotin-labeled mutant oligonucleotides with nuclear extract. Lanes 2–8 contained oligonucleotides with consensus binding sites for different transcription factors with fold concentrations as indicated.

control, and not found in the dbSNP databases were subjected to segregation analysis. As only 0.3% of Chinese develop psoriasis, it is possible that a fully penetrant 17q variation with minor allele frequencies as high as 1–5% could result in a disease phenotype. Therefore, we have also performed segregation analysis in all of the 11 variants found in the dbSNP database, either with minor allele frequencies $<5\%$ (two variants, 4 and 1.2%) or with unknown frequencies (nine variants).We found that none of these 11 variants segregated with the disease in the family (data not shown).

The p.Ala201Val variant of *CD7* was completely cosegregated with the disease in the psoriasis family. The normal controls carrying this variation also show symptoms of allergy or skin disease. However, this variation does not affect either CD7 expression on T and NK cells or the costimulatory activity of CD7 by comparing PBMCs of patients and controls. This may be due to the fact that the amino-acid change from alanine to valine seems to be conservative and non-deleterious. The p.Ala201Val variant lies in the predicted transmembrane domain of the CD7 molecule and may not affect the structure and function of the transmembrane domain on the CD7 molecule. We have also constructed stable transfectants from a CD7-negative T cell line, H9, with the normal or mutant *CD7* gene integrated in the chromosome by retrovirus. The stable transfectants were used to further examine the CD7 expression, costimulatory activity, and galectin-1-mediated death through CD7, because the binding of extracellular galectin-1 and galectin-3 to CD7 induced apoptosis in T cells (CD7, CD43, and CD45 as galectin-1 receptors, whereas CD7 and CD29 as galectin-3 receptors) (Pace *et al.*, 1999; Fukumori *et al.*, 2003; Elola *et al.*, 2005). No significant change was found in the known function of CD7 (data not shown). Our data, combined with the studies in PBMCs from patients and controls (Figure 2), suggested that *CD7* is unlikely to be a disease-causing gene for psoriasis.

ZNF750, which encodes a putative C_2H_2 zinc-finger protein, was first identified as the cause of seborrhea-like dermatitis with psoriasiform elements in an Israeli Jewish Moroccan family (Birnbaum *et al.*, 2006). Hyperkeratosis of skin over the elbows, knees, palms, and soles is evident, and the affected members also present with seborrhea-like erythematous rash on sebaceous follicle-rich areas. Affected members carry a mutation (c.56_57dupCC) that causes a frameshift leading to a truncated protein. Because the mutant transcript is expressed in the keratinocytes of affected individuals and not in the normal control, and also because affected individuals have a high level of *ZNF750* transcript, a dominant-negative model was proposed. Although we identified *ZNF750* as the putative gene responsible for psoriasis in our five-generation family, our study differs from that of Birnbaum *et al.* (2006) both in clinical presentation and in molecular mechanism. First, although the clinical manifestation in our psoriasis family varies from mild lesions on the elbows and knees to the classical plaque over most of the body surface, there is no associated seborrheic dermatitis. Second, at the molecular levels, the mutation we identified is a point mutation in the promoter region, which decreases the promoter activity of *ZNF750*, thus suggesting a haploinsufficiency model.

Attempting to measure the mRNA levels of *ZNF750* in the keratinocytes, where the gene is highly expressed, was not possible because the family refused to participate in skin biopsies. The *ZNF750* transcripts in the Epstein–Barr virus-transformed lymphoblastoid cells of patients and controls were measured by TaqMan quantitative PCR with an Assay-on-Demand kit from Applied Biosystems (Foster City, CA); however, the levels of the transcripts were quite low in both normal and patients' cells with the C_t (threshold cycle) value above 34 (data not shown), making the detection of any significant difference unreliable.

The c.−625A>C mutation of *ZNF750* cosegregated with the disease in the psoriasis family and decreased *ZNF750* promoter activity, indicating the involvement of *ZNF750* in psoriasis susceptibility. EMSA data suggested that the c.−625A>C mutation causes an increase in repressor binding to inhibit *ZNF750* promoter activity and is likely to involve the transcription factors ESE-1, ESE-2, and PU.1. It is interesting to note that ESE-1 and ESE-2 are highly expressed in epithelial cells. Further study will be needed to confirm the role of the Ets family in the regulation of the *ZNF750* gene expression. The c.−625A>C mutation was also found in one of 85 sporadic psoriasis patients studied but not in 188 normal subjects; together, the mutation accounts for 1.7% (CI: 0.2–5.84%) of the psoriasis in the Chinese population.

We have also attempted to investigate whether other mutations in the *ZNF750* gene play a role in the more common form of psoriasis. Although we identified three additional sequence variations (c.−646G>A, c.−597C>T, and c. 1046T>C (p.Leu349Pro)), we did not know the functional significance of these variants. Furthermore, the study was limited because we did not fully sequence the *ZNF750* gene in controls, so most variants unique to controls would have been missed. The impact of the *ZNF750* gene in the common form of psoriasis remained to be elucidated.

MATERIALS AND METHODS

Patients

A five-generation psoriasis kindred with apparent autosomal-dominant inheritance was identified at the National Taiwan University Hospital. This family comprised a total of 16 members with classical psoriasis lesions, 10 with mild skin lesions, and 43 who were unaffected, as previously described (Hwu *et al.*, 2005). In addition, 35 independent familial psoriasis and 85 sporadic psoriasis patients were recruited from the Chang Gung Memorial Hospital (CGMH) Health System. Most of the psoriasis families were small with only two members affected; however, eight of them had an extended family with more than four members affected. All individuals were examined by at least two dermatologists. As controls, 188 healthy Chinese subjects were randomly selected from the Taiwan Han Chinese Cell and Genome Bank (Pan *et al.*, 2006). The random Chinese controls and all participating patients were of Han Chinese origin residing in Taiwan. The study was approved by the National Taiwan University Hospital and CGMH institutional review boards. Informed consent was obtained from each participant for all studies in accordance with institutional requirements and the Declaration of Helsinki Principles.

Strategy to identify the disease-causal variants in the index family

Our strategy was to use the classical positional cloning approach by sequencing of candidate genes in the 3.8-Mb region on chromosome 17q to identify the disease-causing gene. Sequence variants present in the proband (individual IV-5 in the five-generation family; Hwu *et al.*, 2005) but not in the normal control (not from this family) and not found in the dbSNP databases were subjected to segregation analysis, which consisted of sequencing all 23 affected members (13 severely affected and 10 mildly affected) and 16 unaffected members in the index family. We considered the disease-causal variants to be segregated completely with the disease and should not be present in normal controls. Functional studies were then carried out to further characterize the mutation.

DNA sequence analysis

Genomic DNA was purified from peripheral blood using the PUREGENE DNA purification kit (Gentra Systems, Minneapolis, MN). For each candidate gene, we sequenced all exons, including 5′ and 3′ untranslated regions, intron–exon boundaries, and up to 1.2 kb of the promoter region. PCR primers were designed by the Primer Z primer design program (http://140.109.54.18/primerz/beginDesign.do) and the Primer 3 program (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). The PCR products were purified with enzymatic ExoI/SAP treatment (USB, Cleveland, OH) and then sequenced using an ABI PRISM 3700 DNA Analyzer (Applied Biosystems). Results were analyzed using Sequencher 4.6 software (Gene Codes Corporation, Ann Arbor, MI), and the DNA sequences obtained were compared to published sequences. Mutation nomenclature follows the journal guidelines (http://www.hgvs.org/mutnomen) with the numbering using the A of the ATG translation initiation codon in cDNA reference sequence as nucleotide +1.

Functional analysis of the *CD7* gene

Flow cytometric analysis of CD7. PBMCs were isolated by Ficoll–Paque (Amersham Pharmacia, Uppsala, Sweden) density centrifugation from heparinized venous blood samples. Staining of cells for three-color flow cytometry was performed. Briefly, 5×10^5 cells were suspended in 100 µl of phosphate-buffered saline containing 0.1% sodium azide and 3% fetal calf serum and were incubated with different fluorochrome-conjugated (FITC, phycoerythrin, or allophycocyanin (APC)) mAbs for 20 minutes at 4 °C, including CD3 (UCHT1), CD4 (RPA-T4), CD7 (M-T701), CD8 (RPA-T8), and CD56 (B159) purchased from BD Pharmingen (San Diego, CA). Appropriate fluorochrome-conjugated mAbs of the same isotype were used as controls. The stained cells were washed three times, fixed in 1% paraformaldehyde in phosphate-buffered saline, and analyzed for immunofluorescence using a FACSCalibur flow cytometer (Becton-Dickinson, San Jose, CA). The level of CD7 expression on $CD4^+$, $CD8^+$, and $CD56^+$ subsets was determined by multicolor staining followed by FACS analysis.

T-cell activation. Round-bottom 96-well plates were precoated with 200 µl 10 µg ml^{-1} anti-CD3 (UCHT1) and/or anti-CD7 mAb (M-T701) in phosphate-buffered saline and incubated overnight at 4 °C. Purified PBMCs (2×10^5 per well) were cultured in RPMI 1640 plus 10% fetal bovine serum in a total volume of 200 µl for 12, 24, 48, and 72 hours. After culture, the culture supernatant were collected and analyzed for TNF-α and IFN-γ using specific capture ELISA kits (BD Pharmingen), according to the manufacturer's description. The standard curve of each cytokine was performed using a known concentration of recombinant cytokines. For proliferation assays, cells were cultured in plates coated with anti-CD3 and/or anti-CD7 for 24 hours and then pulsed with 1 µCi ^{3}H-thymidine (Perkin Elmer Life Inc., Boston, MA) for another 16 hours. The cells were harvested and the incorporation of ^{3}H-thymidine was measured on a TopCount Microplate Scintillation Counter (Packard, Meriden, CT).

Functional promoter analysis of *ZNF750* gene

Transcription factor binding site identification. The promoter region of *ZNF750* was analyzed with TFSEARCH and MOTIF Search programs based on TransFac database to predict the binding site of transcription factors.

Reporter constructs. The *ZNF750* cDNA reference sequence was derived from GenBank, NM_024702.2. The *ZNF750* promoter sequence was retrieved from the genomic reference sequence with GenBank accession number NT_010663.14. All promoter polymorphisms numbering follows the journal guidelines (http://www.hgvs.org/mutnomen) using +1 as the A of the ATG translation initiation codon. An approximately 0.87-kb fragment (−841 to +31) containing the promoter region of the *ZNF750* was amplified from human genomic DNA and cloned into the pGEM-T Easy vector (Promega, Madison, WI) (Table S1). The C allele of c.−625A>C variant was introduced into the promoter region using site-directed mutagenesis with recombinant PCR method (Ansaldi *et al.*, 1996). These normal and mutated fragments were then released from the pGEM-T Easy vector and subcloned into the upstream region of the firefly luciferase gene of the pGL3-basic vector (Promega). All constructs were subjected to nucleotide sequencing to confirm their correct sequence and orientation.

Transient transfection and luciferase assay. Human keratinocyte cell line, HaCaT, was kindly provided by Dr Te-Chang Lee (Academia Sinica, Taipei, Taiwan) with permission from Dr NE Fusenig (German Cancer Research Center, Heidelberg, Germany). HaCaT cells were grown in DMEM and 10% fetal bovine serum supplemented with 100 U ml^{-1} penicillin, 100 µg ml^{-1} streptomycin, and 2 mM L-glutamine. A total of 2×10^4 cells were seeded in each well of a 24-well plate and transfected with 480 ng of each reporter construct along with 16 ng of pRL-TK vector (Promega) containing the *Renilla* luciferase gene as an indicator for normalization of transfection efficiency. Transfections were performed by using FuGENE HD (Roche Applied Science, Indianapolis, IN), according to the manufacturer's instructions. Cells were incubated for 48 hours and analyzed for luciferase activity with the Dual-Luciferase Assay System (Promega). Firefly luminescence was normalized to *Renilla* luminescence and reported as relative luciferase units. All experiments were performed in triplicate and independently performed at least three times.

Electrophoretic mobility shift assay. Nuclear extracts from human HaCaT cells were prepared using the Nuclear Extract Kit (Active Motif, Carlsbad, CA), according to the manufacturer's instructions. Oligonucleotides were synthesized and biotin-labeled using the Biotin 3′ End DNA Labeling Kit (Pierce Biotechnology,

Rockford, IL). The sequence of the biotin-labeled probes are as follows: N1 5′-TGCTCAAGTTCTAAAGGAAAGTCTCACATG-3′ and M1 5′-TGCTCAAGTTCTAACGGAAAGTCTCACATG-3′. We incubated HaCaT cell nuclear extract and biotin-labeled double-strand oligonucleotide using a non-radioactive LightShift Chemiluminescent EMSA Kit (Pierce Biotechnology). The reaction was carried out with 5 μg nuclear proteins and 1 μg of poly(dI-dC) at 25 °C for 20 minutes. For competition studies, we incubated a 5- to 100-fold excess of unlabeled oligonucleotides (29–30 bp in length) with the nuclear extract before adding the biotin-labeled oligonucleotide. The protein–DNA complexes were separated from unbound DNA on a non-denaturing 8% polyacrylamide gel and transferred onto a nitrocellulose membrane. We detected the signal with LightShift Chemiluminescent EMSA Kit (Pierce Biotechnology), according to the manufacturer's instructions. The sequences of oligonucleotides containing consensus binding sites for transcription factors for competition studies were listed in Table S1.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Table S1. Primers for reporter construct and EMSA.

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